In vivo ^{133}Cs-NMR a probe for studying subcellular compartmentation and ion uptake in maize root tissue

Three ^{133}Cs-NMR signals were observed in the spectra of CsCl-perfused and CsCl-grown maize seedling root tips. Two relatively broad lower field resonances were assigned to the subcellular, compartmented Cs^+ in the cytoplasm and vacuole, respectively. The rate of area increase of the broader cytoplasmic Cs resonance was about 9-times faster than that of the vacuolar signal during the first 300 min of tissue perfusion with CsCl. In addition, the spin lattice relaxation time of the cytoplasmic Cs resonance was approx. 3-times shorter than that of the extracellular resonance, while the Cs^+ signal associated with the metabolically less active vacuolar compartment exhibited a relaxation time comparable to that of the extracellular signal. ^{133}Cs spectra of excised, maize root tips and excised top sections of the root adjacent to the kernel, each grown in 10 mM CsCl showed a difference in the relative areas of the Cs resonances corresponding to the distinct cytoplasm/vacuole volume ratio of these well differentiated sections of the root. The high correlation of counterion concentration with ^{133}Cs chemical shifts suggested that the larger downfield shift exhibited by the cytoplasmic confined Cs^+ was due principally to the higher ionic strength and protein content in this compartment. Such observations indicate that ^{133}Cs-NMR might be employed for studying ionic strength, and osmotic pressure associated chemcial shifts and the transport properties of Cs^+ (perhaps as an analogue for K^+) in subcellular compartments of plant tissues.

Introduction

To date, no spectroscopic methodology is available to directly resolve or visualize the intracellular distribution of metal ions, i.e., the partitioning between subcellular compartments such as the cytoplasm and vacuole in living plant tissues. Information of this kind, especially for an important ion like K^+, could prove invaluable because its compartmentation bears on the questions concerning the accommodation of the plant to osmotic stress [1], the regulation of intracellular pH [2] and the control of membrane transport and metabolic processes [2]. Previous NMR studies of metal ion uptake in maize root tips have taken advantage of the indirect observation of ^{31}P resonance broadening caused by Mn^{2+} trapped in the plant cell vacuole [3,4]. In those experiments, the pH-dependent ^{31}P chemical shift allowed for simultaneous observation of orthophosphate corresponding to the cytoplasmic P_i resonance (pH 7.6) and preferentially broadened vacuolar P_i resonance (pH 5.5) in the intact cells. Thus, the trapping of the paramagnetic Mn^{2+} in the cell vacuole indirectly defined the compartmented site of this ion. Shift reagents, on the other hand, have been used widely to estimate directly the intracellular/extracellular distribution of metal ions such as Na^+ and K^+ in erythrocytes, yeast and mammalian cells [5] as well as plant tissues [6,7]. However, shift reagents are limited by their instability to tissue phosphatase [8] and may themselves in certain instances contaminate the intracellular environment of the tissue [9,10]. To date, subcellular compartmentation of metal ions has never been observed in living plant cells [11]. The available information on compartmental analysis of K^+ or Na^+ in plant cells, for example, has only been surmised through indirect kinetic measurements [6,7]. In general, direct observation of K^+, Na^+ or Rb^+ by NMR is hindered by their high degree of magnetic resonance invisibility due to inherently strong quadrupolar lattice interactions observed in vivo [5]. Quantum filtering methods have helped to visualize

Correspondence: P.E. Pfeffer, Plant and Soil Biophysics, U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118, U.S.A.

these normally invisible transitions; however, the spectral sensitivity is significantly diminished [12,31].

In 1969, it was reported by Halliday and co-workers [13] that the ^{133}Cs chemical shift was sensitive to the nature and concentrations of its counterion, but insensitive to the presence of other cations. Recently, Davis et al. [14] exploited this property of ^{133}Cs in their studies on the uptake of CsCl in red blood cells and perfused heart. In essence, these experiments demonstrated that without the use of shift reagents, one could observe the low field shifted Cs resonance corresponding to intracellular Cs^+ relative to the resonance representing Cs^+ external to the cell. In addition, they demonstrated that Cs^+, like Rb^+ [15], exhibited transport properties akin to potassium ions. The authors attributed the difference in chemical shift between the intracellular and extracellular Cs resonance primarily to the high cellular concentration of phosphate which was capable of inducing twice the shift as chloride ions on the Cs resonance at pH 7.0 [14]. Since ^{133}Cs has inherently small quadrupolar lattice interactions, as evidenced by its relatively long relaxation times, excellent NMR visibility and quantitation of this ion were also obtained [14].

In connection with our ongoing NMR studies of ion transport in root tissue [16–18], we developed a need to understand the exact nature of ion compartmentation within functioning cells. In this report, we describe the first direct NMR observation and assignment of both cytoplasmic and vacuolar resonances representing subcellular compartmented Cs^+ in living plant tissue.

Materials and Methods

Plant tissue

Maize (Zea mays BF-43) root tips (approx. 700, 3–5 mm) were excised from 3-day-old seedlings grown at 27 °C in trays lined with paper soaked with 0.1 mM $CaCl_2$. For experiments used to determine compartmentation of CsCl, the 3-day-old seedlings were grown in trays, as above, containing 0.1 mM $CaCl_2$ and 10 mM CsCl. Excised tips (3–5 mm) and stem sections just below the kernel (3–5 mm) were harvested for experiments to establish the identity of the two resonances representing the cytoplasmic and vacuolar compartmented Cs.

Cell walls from approx. 900 maize root tips (3–5 mm) were prepared as previously described [19]. This preparation was stirred overnight with 0.5 mM EDTA to remove Ca^{2+}, washed, and then incubated with 10 mM CsCl for 3 h before its examination by NMR.

Phosphate, both soluble and insoluble, was determined in root tips and upper segments according to the method of Fiske and SubbaRow [20]. The chloride determination was carried out via precipitation of AgCl as described by Hillerbrand et al. [21].

NMR experiments

All spectra were obtained with a JEOL GX 400 NMR spectrometer operating at 9.4 T corresponding to a ^{133}Cs-NMR frequency of 52.3 MHz and a ^{31}P frequency of 161.7 MHz. ^{31}P spectra were routinely obtained prior to and following the examination of the ^{133}Cs spectra to verify the energetic state of the perfused tissue. The perfusion system, which included 1000 ml of perfusion medium, was essentially the same as previously reported as were the acquisition parameters used to obtain the 27 min ^{31}P spectra [4]. A state of hypoxia was created by bubbling N_2 as opposed to O_2 through the perfusion medium for approx. 1.5–2 h [16]. The aerobic and hypoxic state of the tissue was established from ^{31}P spectra taken during the initial and final periods of the experiments [16]. ^{133}Cs spectra were obtained with either a 90° pulse (27.4 μs) and a pulse delay of 30 s or with a 60° pulse (17 μs) and a pulse delay of 22 s to give quantitative responses for all resonances. Spectral widths were 1000 Hz, with 4k data points and either 10 or 3 Hz digital line broadening. Each of the intracellular resonance areas was quantified with a (Gauss-Newton) non-linear regression curve fitting routine (see Fig. 2). In Cs influx studies, each spectrum obtained from 32 transients was separated in a stacked experiment by a 1 h waiting period (see data given in Fig. 2). Following the completion of the experiment (approx. 12–20 h), the accumulated free induction decays (FID) were stored on disk, normalized to the FID of the first spectrum, Fourier transformed and printed out in sequence. The area of each resonance was evaluated as indicated above. Initial rates of Cs influx were calculated from the slopes of the Cs^+ curve over the first 300 min of perfusion. All spectra were obtained with the spectrometer in an unlocked mode.

The percent visibility of ^{133}Cs by NMR was evaluated on the basis of the area of the ^{133}Cs resonance representing the external 10 mM CsCl. The volume represented by this free circulating solution within the coil volume was 0.35 ml. The area of the external ^{133}Cs resonance was measured without perfusion because the flow of liquid gave an underestimation of area by 10%. The net fresh weight of the excised tips within the coil volume was approx. 1 g. The determination of total Cs within the tips was made with a Perkin-Elmer atomic absorption spectrophotometer and a Cs hollow cathode lamp. The values found for NMR and atomic absorption measurements were 29 ± 2 and 31 ± 3 μmol/g fresh weight, respectively.

Spin lattice relaxation (T_1) measurements were performed by the inversion recovery method (180°–τ–90°) with and without perfusion to determine the effect of perfusion on the T_1 of the Cs in the circulation medium.

Acquisition parameters were as above, with a 90° pulse of 27.4 μs, and delay between acquisitions of 50 s (48 s pulse delay and 2 s acquisition). The data were evaluated by a three-parameter non-linear regression analysis.

In vitro ^{133}Cs chemical shift experiments were performed using a 10 mm dual concentric tube assembly [22]. The exterior section contained 10 mM CsCl and the interior tube contained 10 mM CsCl plus the additional solute to be added i.e., KCl, KH_2PO_4, K_2HPO_4, KNO_3, or ovalbumin at pH 3, 4.6 and 7.5. The acquisition parameters used were as described above for the in vivo experiments. Differences in chemical shift due to magnetic susceptibility between inside and outside solutions were negligible relative to measured Cs shifts.

Results

Fig. 1 shows four representative ^{133}Cs spectra from a 20 h aerobic influx study of excised maize root tips that had been perfused with 50 mM glucose and 10 mM CsCl, buffered at pH 6.0 with 10 mM Mes under aerobic conditions. Following curve fitting the least

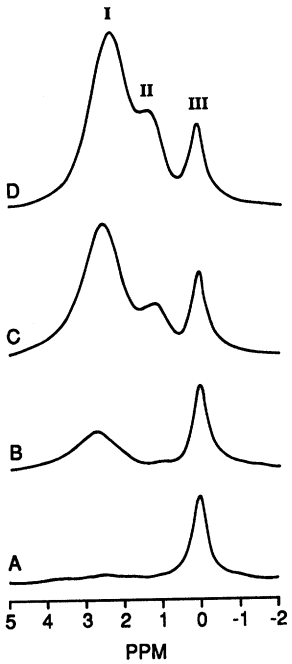

Fig. 1. 52.3 MHz ^{133}Cs-NMR spectra of approx. 600–900 excised (3–5 mm) maize (*Zea mays* BF-43) root tips from 3-day-old seedlings perfused with 50 mM glucose, 10 mM CsCl, 0.1 mM $CaSO_4$ buffered with Mes and Bistris propane to pH 6.0. Acquisition parameters; 4k data points, spectral width = 100 Hz, 90° pulse = 27.4 μs, pulse delay 30 s, 32 transients per spectrum, exponential line broadening 10 Hz. (A) 19 min after adding 10 mM CsCl to the perfusion medium; (B) 1 h 30 min after perfusion with the medium containing 10 mM CsCl; (C) 7 h 45 min of perfusion; and (D) 15 h 15 min of perfusion. The resonance at 0.0 ppm corresponds to the Cs^+ in the perfusion medium and the resonances at 1.08 and 2.35 ppm represent intracellularly compartmented Cs^+. All spectra were obtained under quantitative conditions to avoid intensity distortions due to differences in T_1. Peaks I, II and III are described in the text.

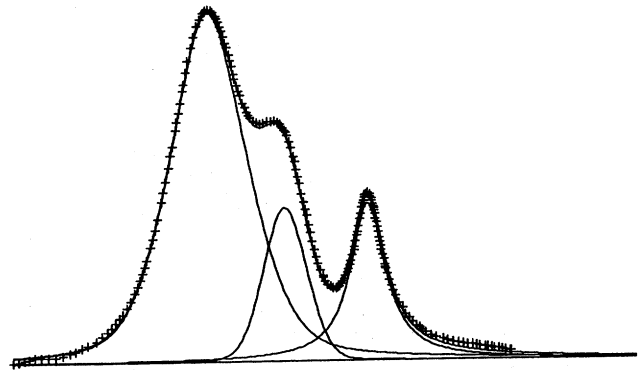

Fig. 2. Non-linear regression analysis curve fit of a digitied 52.3 MHz ^{133}Cs spectrum of maize root tips followed approx. 20 h of perfusion with 10 mM CsCl at 21° C in Fig. 1.

shielded resonance (I) at approx. 2.35 ppm was found to be considerably broader (42 Hz) than the higher field resonance (II) (27 Hz) at approx. 1.08 ppm (see Materials and Methods). The resonance linewidth of peak (III), eminating from the external circulating 10 mM CsCl solution at 0.00 ppm was approx. 11 Hz (the spectra shown contain 10 Hz computer line broadening). These linewidths appeared to remain relatively constant through the duration of this experiment. In contrast, under hypoxic conditions i.e., perfusion with an N_2 saturated solution of 10 mM CsCl, no resonances corresponding to I and II appeared in time periods up to 20 h. However, a small broad resonance at approx. 0.5 ppm became evident within 8 h (data not shown). Based on the known concentration of Cs^+ in the perfusate, the volume of the perfusion medium (corrected for the volume occupied by the roots) and the fresh weight of roots centered within the coil, we calculated from the ratio of the peak areas that the root tissue absorbed a maximum of 18 μmol Cs^+ per g of wet tissue in 20 h. Of this, 3.7-times as much Cs^+ resided in the environment characterized by peak I as compared with peak II. Fig. 2 shows a typical digitized and curve fit ^{133}Cs spectrum of maize root tips following approx. 20 h of perfusion with 10 mM CsCl. To assure that all the resonance ratios were undistorted, spectral recycling was set equal to 5-times the longest T_1 (to be discussed below). NMR visibility of the influxed Cs^+ was evaluated by comparing the quantity measured by NMR and that obtained from atomic absorption measurements. These values agreed within 7%, with the value for the atomic absorption being somewhat higher.

^{31}P spectra of the perfused tissue were obtained prior to and following the addition of CsCl to the oxygenated perfusion medium to evaluate the status of the tissue (data not shown). These spectra showed the continued maintenance of the intracellular pH gradient between the cytoplasm and vacuole, relatively high levels of NTP and little evidence of possible Cs toxicity. Generation of vacuolar phosphate emanating from nucleotide and

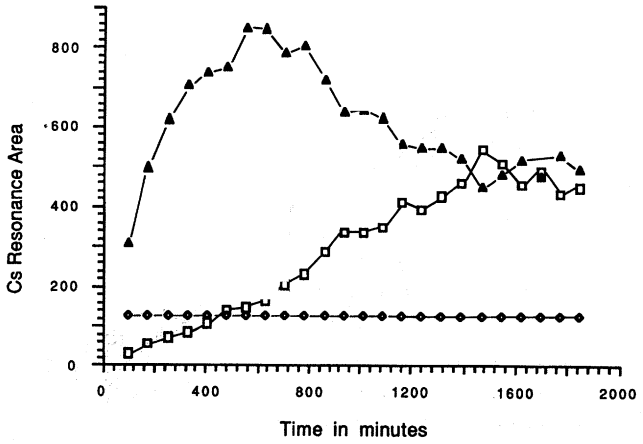

Fig. 3. Plot of influx of partitioned intracellular Cs^+ in excised maize root tips as described in Fig. 1, and circulating external Cs^+ (1 l of 10 mM CsCl), as a function of perfusion time with circulating buffer, as mentioned in Fig. 1. Initial Cs^+ influx rates were calculated from the first 300 min of perfusion. Curve ▲ represents the low field intracellular compartmented Cs^+ resonance (Peak I); □ the higher field intracellularly compartmented Cs^+ resonance (Peak II); and ◆ represents the resonance area of the external Cs^+ (Peak III).

membrane degradation over the 20 h period was consistent with our previous observations [16]. A slight broadening of the cytoplasmic P_i resonance suggested that some pH control mechanisms may have been disturbed because of depletion of K^+. To ensure that neither shifted Cs resonance corresponded to a cell-wall-bound ion, Ca^{2+}-free cell walls obtained from maize root tips were incubated with 10 mM CsCl. No shifted Ca resonance was evident in the presence of these cell wall preparations.

Fig. 3 shows the uptake curve of Cs^+ in the two intracellular compartmented sites characterized by resonances I and II as well as resonance III (referenced as 0.00 ppm, represented the externally perfusing 10 mM CsCl). For the first 300 min, the average rates of Cs^+ uptake of sites I and II were 74.5 nmol/g fresh wt. per min and 8.0 nmol/g fresh wt. per min, respectively. The uptake of the slower compartment (II) exhibited a lag period of approx. 1 h before significant influx was observed. However, it was difficult to judge exactly how long this lag was, since the initial intensity of resonance II was very weak. Following 10 h of perfusion the low field resonance I represented a 6-fold larger area or quantity of Cs^+ than II. The sigmoidal uptake exhibited in compartment I leveled off at approx. 20 h after going through a maximum. Compartment II continued to take up Cs^+ steadily until it leveled off at 23 h.

Fig. 4 shows the ^{133}Cs spectra of excised maize 3–5 mm root tips and top stem sections (3–5 mm), adjacent to the kernel, of 3-day-old seedlings grown on 10 mM CsCl. For simplicity, no CsCl was added to the perfusion medium. In Fig. 4A the low-field intracellular resonance representing compartment I of root tips is observed at 2.35 ppm and Cs in compartment II at 1.05 ppm. The ^{133}Cs spectrum of the corresponding top root sections adjacent to the kernel is seen in Fig. 4B. Here the lower field broad intracellular Cs resonance was observed at 2.10 ppm, while the somewhat sharper resonance was observed at 0.77 ppm. The partial ^{31}P-NMR spectrum showing the downfield resonance of the phosphomonoesters centered at approx. 5.0 ppm, cytoplasmic P_i at 2.6 ppm and the vacuolar P_i at 0.9 ppm of each root section is shown as an insert next to its corresponding ^{133}Cs spectrum. The area ratio of the low-field to high-field Cs resonance (Fig. 4A), obtained from a non-linear regression curve fitting analysis of the spectrum for the root tips was 3.63, while the corresponding ^{31}P resonance ratio for cytoplasmic/vacuolar P_i was found to be 1.5. The ^{133}Cs spectrum of the top section of the root tissue 4B exhibited a low-field to high-field resonance ratio of 0.25 and the corresponding ^{31}P resonance ratio of the cytoplasmic/vacuolar P_i was less than 0.09.

^{133}Cs spin lattice relaxation times (T_1), as measured

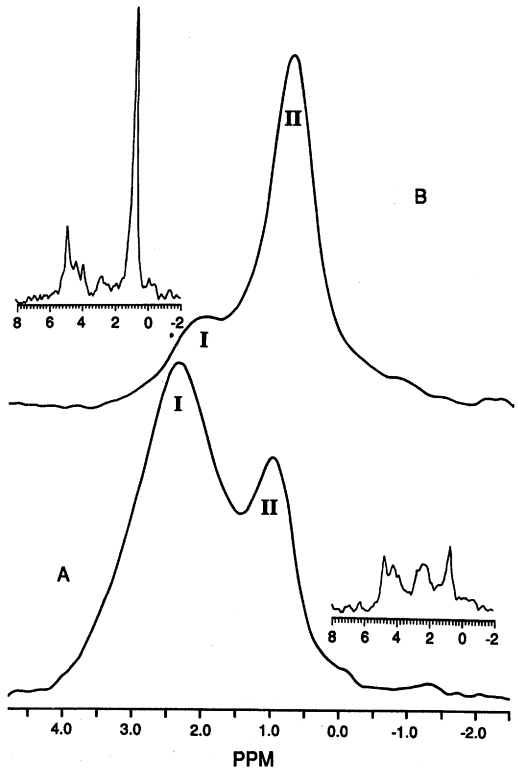

Fig. 4. (A) 52.3 MHz ^{133}Cs-NMR spectrum of approx. 700 3-day-old excised maize root seedling tips (3–5 mm) grown in 0.1 mM $CaSO_4$, and 10 mM CsCl solution over wet filter paper and (B) root top sections below the kernel (3–5 mm) grown as in (A). Both spectra were obtained with a 60° pulse, 22 s delay, a spectral width of 1000 Hz, 4k data points and 10 Hz digital line broadening. The ^{31}P-NMR spectra (insets) of the corresponding root sections were obtained as described previously [4]. The resonances at approx. 2.6 ppm represent P_i in the cytoplasmic compartment and the resonance at approximately 0.9 ppm represent vacuolar P_i. All the tissue was continually perfused during data acquisition [4].

TABLE I

NMR properties of ^{133}Cs resonances in excised maize root tips perfused with 10 mM CsCl (in vivo) or in a solution of 10 mM CsCl with or without protein (in vitro)

	In vivo			In vitro	
	I	II	III	with 20% ovalbumin	without ovalbumin
Relaxation time T_1 in s	1.91 ± 0.04	6.09 ± 0.09	6.12 ± 0.23	5.50	11.60
Chemical shifts in ppm	2.35	1.08	0	–	–
Linewidth in Hz	42	27	11	2.6	0.75

by the inversion recovery method, indicated that all the signals recovered with a single exponential time constant. The T_1 values, linewidths and chemical shifts for the intracellular and extracellular resonances are given in Table I. In vitro, the linewidths of the Cs resonances, in the absence of plant tissue, but with and without 20% ovalbumin were 2.6 Hz and 0.75 Hz, respectively, and the corresponding T_1 values were 5.50 s and 11.60 s, respectively. As we see, the presence of root tips or tops (heterogeneous tissue), or protein can diminish ^{133}Cs T_1 values by 50%.

To examine the possible origin of the Cs chemical shift within the plant tissue, the influence of anion concentration and temperature on the resonance was investigated in vitro. Fig. 5 shows the influence of different anion concentrations on the ^{133}Cs resonance position. The most abundant in vivo anion, Cl^- (330–450 μmol/g dry wt.) produced an average shift of 0.015 ± 0.001 ppm/mmol at 21° C up to a concentration of 400 mM KCl. Soluble phosphate, which is present only in concentrations of 90–128 μmol/g dry wt., produced Cs shifts at pH 9.1 (HPO_4^{-2}) of 0.025 ppm/mmol and 0.007 ppm/mmol at pH 4.4 ($H_2PO_4^-$) respectively, up to a concentration of 400 mM. Protein (ovalbumin) over a concentration range from 2.5 to 20% at 21° C produced diamagnetic Cs shifts of 1.75 ppm at pH 3.0, 1.47 ppm at pH 6.2 and 1.76 ppm at pH 8, respectively (data not shown). In addition, temperature also influences the Cs^+ chemical shift, e.g., a 20% ovalbumin solution containing 10 mM CsCl; underwent a −1 ppm shift over a range of 8° C (17–25° C) a shift of −0.126 ppm/degree.

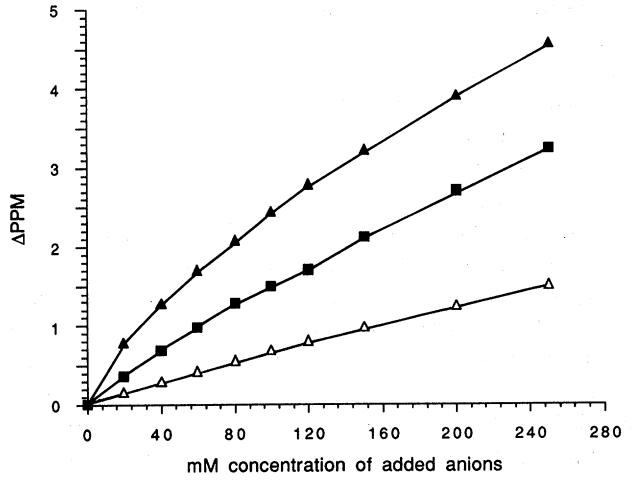

Fig. 5. ^{133}Cs chemical shifts as a function of anion concentration. ▲ HPO_4^{-2} solution (pH 9.1); △ $H_2PO_4^-$ solution pH 4.4; ■ Cl^- solution. In vitro titration experiments were carried out using a 10 mm dual concentric tube assembly [22]. The exterior section contained 10 mM CsCl and the interior tube, of equal volume, contained 10 mM CsCl plus the added ion. Differences in chemical shift due to magnetic susceptibility between inside and outside solutions were negligible relative to the measured Cs shifts.

Discussion

Based on our findings we suggest that the ^{133}Cs resonances at 2.35 ppm (I) and 1.08 ppm (II) represent the Cs^+ in the cytoplasm and vacuole of maize root cells, respectively. These conclusions are supported by the following observations.

(1) In excised root tips perfused with 10 mM CsCl, the initial buildup of the resonance signal is observed in region I. This buildup is presumably a consequence of the transport of the Cs^+ across the plasma membrane into the cytoplasm (see Fig. 1B). The increase in the Cs^+ signal may also constitute and exchange of Cs^+ with a high concentration of K^+ residing in the cytoplasmic compartment [23]. Typically, the ratio of K^+ cytoplasmic/K^+ vacuolar in barley is of the order of approximately 5 : 1 [24]. After a lag time of approx. 1 h, a second Cs^+ resonance (II) became evident in the upfield region of the spectrum (see Fig. 1C). A similar biphasic uptake kinetics of Rb^+, attributable to movement through the plasmalemma and tonoplast membranes, respectively, has been surmised in barley roots [25].

Fig. 3 shows a typical Cs^+ influx curve based on the change in ^{133}Cs resonance areas I and II with time. The intensity of the ^{133}Cs resonance in the cytoplasm first showed a rapid increase and then slowly decreased to a steady level. On the other hand, the increase of Cs^+ in the vacuole appeared to follow a sigmoidal pattern. Three interesting points are noted. First, both signals appeared to reach stable levels at about the same time, approx. 23 h after perfusion. Secondly, the beginning of

the vacuolar Cs^+ increase coincided with the decrease of cytoplasmic Cs^+. And thirdly, the decrease of cytoplasmic Cs^+ from its peak at approx. 10 h to a steady level is about the same as the total increase of vacuolar Cs^+, suggesting a sequential uptake event. While the exact molecular origin of the first two observations remains unknown, a qualitative mechanism may be offered. With the external Cs^+ concentration at 10 mM, we estimated that cytoplasmic Cs^+ reached about 30 mM before decreasing to a stable level of 20 mM. Assuming that the uptake of Cs^+ across both plasma and vacuolar membranes requires either a simple carrier or channel, the sigmoidal kinetics suggests that the K_m of the tonoplast carrier/channel is much higher than that associated with the plasma membrane. The data also suggest that the tonoplast carrier/channel exhibits an induced positive cooperativity which allows the vacuolar uptake rate to exceed that of cytoplasmic uptake. Since both cytoplasmic and vacuolar uptake of Cs^+ reach stable levels, the following rate (R) relationships should hold:

$$RP_i + RV_e = RP_e + RV_i \quad (1)$$

$$RV_i = RV_e \quad (2)$$

$$RP_i = RP_e \quad (3)$$

in which P, V, e and membrane, vacuolar membrane, efflux, and influx, respectively. Eqns. 1 and 2 delineate the required rate relationships for maintaining cytoplasmic and vacuolar Cs^+ at the steady state. A substitution of Eqn. 2 into Eqn. 1 indicates that, at the steady-state level, the efflux and influx across the plasma membrane are equal as well (Eqn. 3). It is interesting to note that unlike the 23 h CsCl-perfused excised maize root tips (Fig. 3), the 3-day-old excised tips derived from seedlings grown on 10 mM CsCl (Fig. 4) do not show an equal distribution of Cs^+ between the cytoplasm and vacuole but rather a ratio of 1.3. This is probably a consequence of the fact that not all the Cs^+ that enters the cytoplasm of the intact seedling moves into the vacuole, i.e., some portion can be exported to the leaves via the xylem. Thus the distribution of Cs^+ in the CsCl grown seedlings represents an equilibrium which is physiologically more relevant.

In the absence of O_2, i.e., hypoxic conditions, the energy derived from oxidative phosphorylation is no longer available to fuel the energetic movement of Cs^+ across the plasmalemma. The small, broad Cs resonance observed at the higher field position (approx. 0.5 ppm) in these spectra (data not shown) most likely represents an accumulation of cell-wall-bound Cs which is unable to pass the boundary of the adjacent plasmalemma. Alternatively, this resonance might represent a small quantity of Cs^+ that has moved into the cytoplasm but has a small chemical shift due to the dimination of cell protein present during hypoxia.

(2) Our in vitro study of ^{133}Cs chemical shift as a function of anion and protein concentration suggested that resonance I of the in vivo spectrum should correspond to the Cs^+ in the environment having the highest ionic strength and protein concentration. The cytoplasm of these roots, which were not grown on phosphate medium contain predominantly Cl^- (> 200 mM) [1] and a relatively minor amount of phosphate, (approx. 6 mM) as has been determined by detailed microscopy and NMR cell volume analysis [26,27]. The total soluble phosphate in the tips was only 128 μmol/g dry wt. whereas chloride was present at the level of 450 μmol/g dry wt. Thus chloride is the dominant counterion responsible for the low field shift of Cs.

There is little protein in the vacuole relative to the cytoplasm [28]. Since protein, on a millimolar basis, induces a greater downfield shift on Cs than simple anions, it is apparent that the protein rich compartment is probably represented by peak I. We suggest that the broadened nature of this resonance is due principally to cellular inhomogeneity (a range of cytosolic ion strengths within the cell population) in addition to chemical exchange among magnetically non-equivalent environments (20% protein produced a 3-fold increase in Cs resonance linewidth, see results above). Consequently, broadening is less evident in peak II since much lower concentrations of metabolically active proteins are found in the vacuole [28].

(3) It is significant that the ^{133}Cs spin lattice relaxation time (6.04 s) of peak II is almost 3-times longer than peak I (1.91 s) whereas T_1 of peak II and the resonance of free extracellular Cs^+ III (in the presence of heterogeneous tissue) are comparable in magnitude. Since the vacuole is a highly disordered compartment with little biochemical activity, one might expect it to resemble the environment of the exterior circulating medium. We have observed as well that the in vitro T_1 of Cs^+ is shortened in the presence of 20% protein from 11.6 to 5.5 s. Thus, protein interactions probably play a major role in shortening the observed in vivo T_1 values.

(4) Based on the area of peaks I and II, which represent the total amount of Cs^+ in the subcellular compartments, we can conclude that the root tip section from corn seedlings (3–5 mm) grown with 10 mM CsCl contains more I than II (I/II = 3.6 : 1), and conversely the upper section of the root adjacent to the kernel cells contains more II than I (I/II = 0.25). It has been reported that the concentration of K in the cytoplasm is considerably higher than that of the vacuole [24]. Taking into account the difference in the average cytoplasm/vacuole volume ratio for the 3–5 mm corn root tips of 40 : 60 [26], we would expect a Cs concentration ratio (cytoplasm/vacuole) of 5.4. Likewise, for the upper

section root cells, a volume ratio of 5 : 95 [29] should yield a Cs concentration ratio of cytoplasm/vacuole of 4.8. It is interesting to note these Cs concentration ratios are similar to the K concentration ratio (5 cytoplasm/vacuole) determined in barley seedling root tips [24]. Note also as described earlier [30], that the ratio of the cytoplasm/vacuolar P_i resonances (see inset in Fig. 4), is a clear indication of the differences in volumes occupied by the vacuole and cytoplasm in each characteristic section of the root.

We are presently systematically exploring the effects of relevant anions and proteins on the chemical shift properties of Cs^+. We anticipate that with this information, ^{133}Cs-NMR can potentially be exploited, as is ^{31}P-NMR, to evaluate intracellular changes in response to environmental stress (e.g., water, salt, hypoxia, phytotoxins). In addition, since Cs^+ does, to a certain degree, mimic K^+ [14] it is quite possible that in vivo ^{133}Cs-NMR will be applicable to the study of K^+ modulated bioenergetic processes involving the regulation of cell osmolarity.

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References

1 Clarkson, D.T. and Hanson, J.B. (1980) Annu. Rev. Plant Physiol. 31, 239–298.
2 Kochian, L.V. and Lucas, W.J. (1988) Adv. Bot. Res. 15, 93–177.
3 Kime, M.J., Ratcliffe, R.G. and Loughman, B.C. (1982) J. Exp. Bot. 33, 670–681.
4 Pfeffer, P.E., Tu, S.-I., Gerasimowicz, W.V. and Cavanaugh, J.R. (1986) Plant Physiol. 80, 77–84.
5 Springer, C.S. (1987) Annu. Rev. Biophys. Chem. 16, 375–399.
6 Sillerud, L.O., Heyser, J.W., Chung, H.H. and Bitewsky, M.W. (1986) inNMR in Living Systems (Axenrod, T. and Ceccarelli, G., eds.), pp. 309–333, Reidel, New York.
7 Gerasimowicz, W.V., Tu, S.-I. and Pfeffer, P.E. (1986) Plant Physiol. 81, 925–928.
8 Martin, J.P., Klein, G. and Satre, M. (1987) Arch. Biochem. Biophys. 254, 559–567.
9 Castle, A.M., MacNab, R.M. and Shulman, R.G. (1986) J. Biol. Chem. 261, 3288–3292.
10 Matwiyoff, N.A., Gasparovic, C., Wenk, R., Wicks, J.D. and Rath, A. (1986) Magn. Reson. Med. 3, 164–168.
11 Belton, P.S. and Ratcliffe, R.G. (1985) Prog. NMR Spectrosc. 17, 241–279.
12 Pekar, J. and Leigh, J.S. (1986) J. Magn. Reson. 72, 159–162.
13 Halliday, J.D., Richards, R.E. and Sharp, R.R. (1969) Proc. R. Soc. Lond. A. 313, 45–69.
14 Davis, D.G., Murphy, E. and London, R.E. ((1988) Biocemistry 27, 3547–3551.
15 Epstein, E. (1972) Mineral Nutrition of Plants: Principles and Perspectives, pp. 325–344, Wiley Interscience, New York.
16 Pfeffer, P.E., Tu, S.-I., Gerasimowicz, W.V. and Boswell, R.T. (1987) Planta, 172, 200–208.
17 Pfeffer, P.E., Tu, S.-I., Gerasimowicz, W.V. and Bosewell, R.T. (1987) in Plant Vacuoles (Marin, B., ed.), pp. 349–354, Plenum, New York.
18 Roby, C., Bligny, R., Douce, R., Tu, S.-I. and Pfeffer, P.E. (1988) Biochem. J. 252, 401–408.
19 Nagahashi, G. and Seibles, T.S. (1986) Protoplasm 134, 102–110.
20 Fiske, C. and SubbaRow, Y. (1926) J. Biol. Chem. 66, 357–400.
21 Hillebrand, W.F., Lundell, G.E.F. and Bright, H.A. (1953) Applied Inorganic Analysis, p. 156, John Wiley and Sons, New York.
22 Pfeffer, P.E., Valentine, K.M. and Parrish, F.W. (1979) J. Am. Chem. Soc. 101, 1265–1274.
23 Marshner, H. (1962) Agrochimica VII, 75–88.
24 Jenschke, W.D. and Stelter, W. (1976) Planta 128, 107–112.
25 Bange, G.G.J. (1978) Acta Bot. Neel. 26, 53–62.
26 Patel, D.D., Barlow, P.W. and Lee, R.B. (1990) Ann. Bot., in press.
27 Lee, R.B., Ratcliffe, R.G. and Southon, J. (1990) Exp. Bot., in press.
28 Lin, W. and Wittenbach (1981) Plant Physiol. 67, 969–972.
29 Loughman, B.C. (1987) Plant Soil 99, 63–74.
30 Roberts, J.K.M., Ray, P.M., Wade-Jardetzky and Jardetzky, O. (1980) Nature 283, 870–872.
31 Allis, J.L. and Rada, G.K. (1989) J. Magn. Reson. 84, 372–375.